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Ploidy and Pronuclear Interaction in Northeastern Pacific *Lasaea* Clones (Mollusca: Bivalvia)

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Abstract. A natural population of the bivalve genus *Lasaea* from Victoria, British Columbia, Canada was karyologically characterized, and pronuclear interaction was studied in newly spawned eggs. Mitotic metaphases from 95 cells were enumerated, and chromosome numbers ranged from 58 to 108, with 90 to 100 being most frequent. Ten well-spread metaphases were karyotyped, and the chromosomes were classified into 32 triplet subgroupings on the basis of shared morphology and size, together with a variable number of supernumerary chromosomes. Northeastern Pacific *Lasaea* clones share broad karyological features with direct developing congeners, but detailed comparisons reveal that they have experienced different evolutionary mechanisms of polyploidy. The pronuclear interaction study generated two key pieces of evidence that establish that northeastern Pacific *Lasaea* clones do not reproduce by self-fertilization, but that parthenogenetic development is triggered by autosperm. The incorporated sperm nucleus disintegrates in the egg cortex and does not fuse with the egg "pronucleus," *i.e.*, syngamy does not occur. Both polar bodies have a diploid chromosome number, a result inconsistent with meiosis, implying that they are products of mitotic divisions. Nonhybridizing lineages of northeastern Pacific *Lasaea* therefore represent true asexual clones, not inbred lines. *Lasaea* is the first bivalve genus in which asexual reproduction has been confirmed and is also the first molluscan genus in which pseudogamy (gynogenesis) has been detected.

Introduction

Lasaea is a near-cosmopolitan genus of minute, hermaphroditic, brooding clams that inhabit crevices in rocky intertidal shores (Keen, 1938; Ponder, 1971; Beauchamp, 1986). We are interested in these organisms because they allow valuable insights into the genetic and evolutionary consequences of alternate life history trait combinations in marine benthic invertebrates. A major reproductive and developmental dichotomy exists within *Lasaea*. One species, *L. australis* (Lamarck, 1818), reproduces by cross-fertilization, releases its progeny as planktotrophic larvae (Ó Foighil, 1988; Tyler-Walters and Crisp, 1989) and is restricted in its distribution to the western Pacific (Ó Foighil, 1989). In contrast, the congeners of *L. australis* all release their young as crawl-away juveniles and form a complex grouping of poorly defined systematic status (Ó Foighil, 1989).

Although lacking pelagic larvae, this group of organisms have attained—possibly by rafting—a remarkably extensive collective geographic range, which includes all continents apart from Antartica and a large number of oceanic islands (Ó Foighil, 1989). Population genetic studies of *Lasaea* with this developmental mode have revealed that natural populations are composed of non-hybridizing, frequently sympatric, genetic strains (Crisp *et al.*, 1983; Crisp and Standen, 1988; Ó Foighil and Eernisse, 1988; Tyler-Walters and Crisp, 1989; Tyler-Walters and Davenport, 1990). There is currently no evidence for cross-fertilization in this grouping. Individuals can reproduce in isolation for at least two generations (Crisp *et al.*, 1983), and progeny from pair mating experiments (Ó Foighil and Eernisse, 1988) and from field brooding specimens (Ó Foighil and Eernisse, 1988; Tyler-Walters and Davenport, 1990) preserve maternal protein phenotypes. *Lasaea* strains studied to date in Europe and Kerguelen Island are highly, and variably, polyploid; indeed, they produce a record number of chromosomes for the Class Bivalvia (Thiriot-Quiévreux *et al.*, 1988, 1989). It is not

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clear, however, if polyploidy is linked to this developmental mode, and the evolutionary mechanism of polyploidy in these organisms is obscure.

The general biology of *Lasaea* is known in considerable detail, but one important question still remains to be resolved: what is the reproductive mode of *Lasaea* strains that lack pelagic larvae? This has proven to be surprisingly difficult to answer, and there are lines of evidence for both self-fertile (Ó Foighil, 1987) and asexual reproductive modes (Crisp and Standen, 1988; Thiriot-Quiévreux *et al.*, 1988, 1989; Tyler-Walters and Crisp, 1989). All studied populations of *Lasaea* that lack pelagic larvae are now known to be simultaneous hermaphrodites with a minute male allocation (Ó Foighil and Eernisse, 1988). Preliminary data on sperm-egg interaction are consistent with self-fertilization: both gamete types are spawned simultaneously into the brood chamber: sperm bind to eggs via an acrosomal reaction, penetrate the egg, and two polar bodies are extruded prior to first cleavage (Ó Foighil, 1987). A variety of other data, however, suggests an asexual reproductive mode in which the sperm merely trigger parthenogenetic development (pseudogamy). Individual *Lasaea* frequently express putative heterozygote electromorphs at multiple loci (Crisp and Standen, 1988; Ó Foighil and Eernisse, 1988; Tyler-Walters and Crisp, 1989). These electromorphs, if real, are inconsistent with a self-fertile reproductive mode. Karyological analyses of *Lasaea* strains have failed to find meiotic metaphases, and the presence of odd ploidy numbers and of supernumary chromosomes may render accurate meiosis impossible (Thiriot-Quiévreux *et al.*, 1988, 1989).

The aims of this study were twofold. First, we karyologically characterized northeastern Pacific populations, an approach to understanding how the exceptionally high chromosome assemblages in *Lasaea* strains evolved. Second, we studied pronuclear interaction in newly spawned eggs to distinguish between the two competing hypotheses about the reproductive mode in *Lasaea* strains.

Materials and Methods

Karyology

Specimens of *Lasaea* were sampled at McNeill Bay, Victoria, British Columbia, Canada in October 1989 and air-mailed live to Villefranche-sur-mer for karyotyping. The McNeill Bay population is composed of at least five non-hybridizing genetic strains that can be reliably distinguished only by electrophoretic analyses (Ó Foighil and Eernisse, 1988). Air-mailed specimens were maintained on arrival in aquaria for 10 days and fed cultured microalgae (*Isochrysis galbana*) to stimulate cell divisions.

Specimens were incubated for 12 h in seawater containing 0.005% colchicine, following which the valves of each clam were gently half-opened to allow effective hypotonic treatment (45 min in 0.9% sodium citrate). Subsequent processing involved fixation in freshly mixed absolute alcohol and acetic acid (3:1) with three changes of 20 min duration. During the second fixation step, the bodies were dissected from the valves. Slide preparations were made from 1 to 4 bodies using an air-drying technique (detailed in Thiriot-Quiévreux and Ayraud, 1982). The preparations were stained for 10 min with 4% Giemsa (pH 6.8), and photographs of well-spread metaphases were taken with a Zeiss III photomicroscope.

For karyotyping, chromosomes were cut out of the photomicrographs and were paired on the basis of size and centromere position. Measurement of chromosomes from the best karyotypes were made with a Digitizer (BIT PAD 10, Summa Graphic) interfaced with a microcomputer (ATV 286). Statistical interpretations were made using a CHROMOS program (Thiriot-Quiévreux, 1984; Thiriot-Quiévreux *et al.*, 1988). Terminology relating to centromere position follows that of Levan *et al.* (1964). When a centromere position was borderline between two categories, the mean was calculated with 95% confidence limits, and both categories were listed.

Pronuclear interaction

Additional animals were sampled from the McNeill Bay in February 1990 for the pronuclear interaction study. Gravid individuals were cultured in petri dishes containing seawater at room temperature and checked daily using a dissecting stereomicroscope for evidence of spawning activity. Newly spawned individuals were detected by the presence of eggs in the brood chamber, an event visible through the semi-transparent valves of most adults. Fifty-four early broods (uncleaved eggs—4 cell stage) were dissected from the parents and individ-

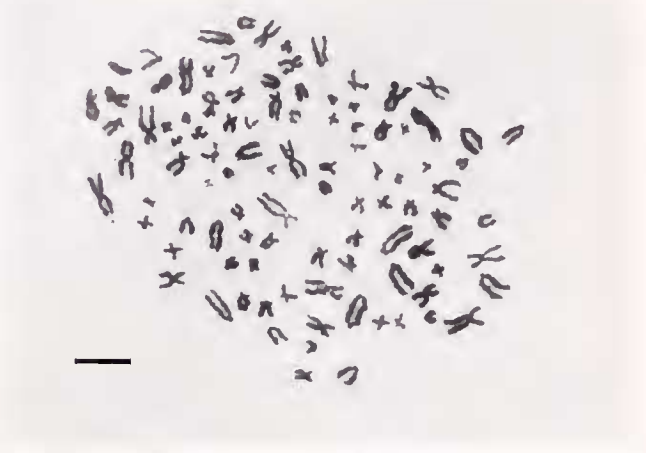


Figure 1. Mitotic metaphase with 100 chromosomes in northeastern Pacific *Lasaea*. Scale bar = 10 μm.

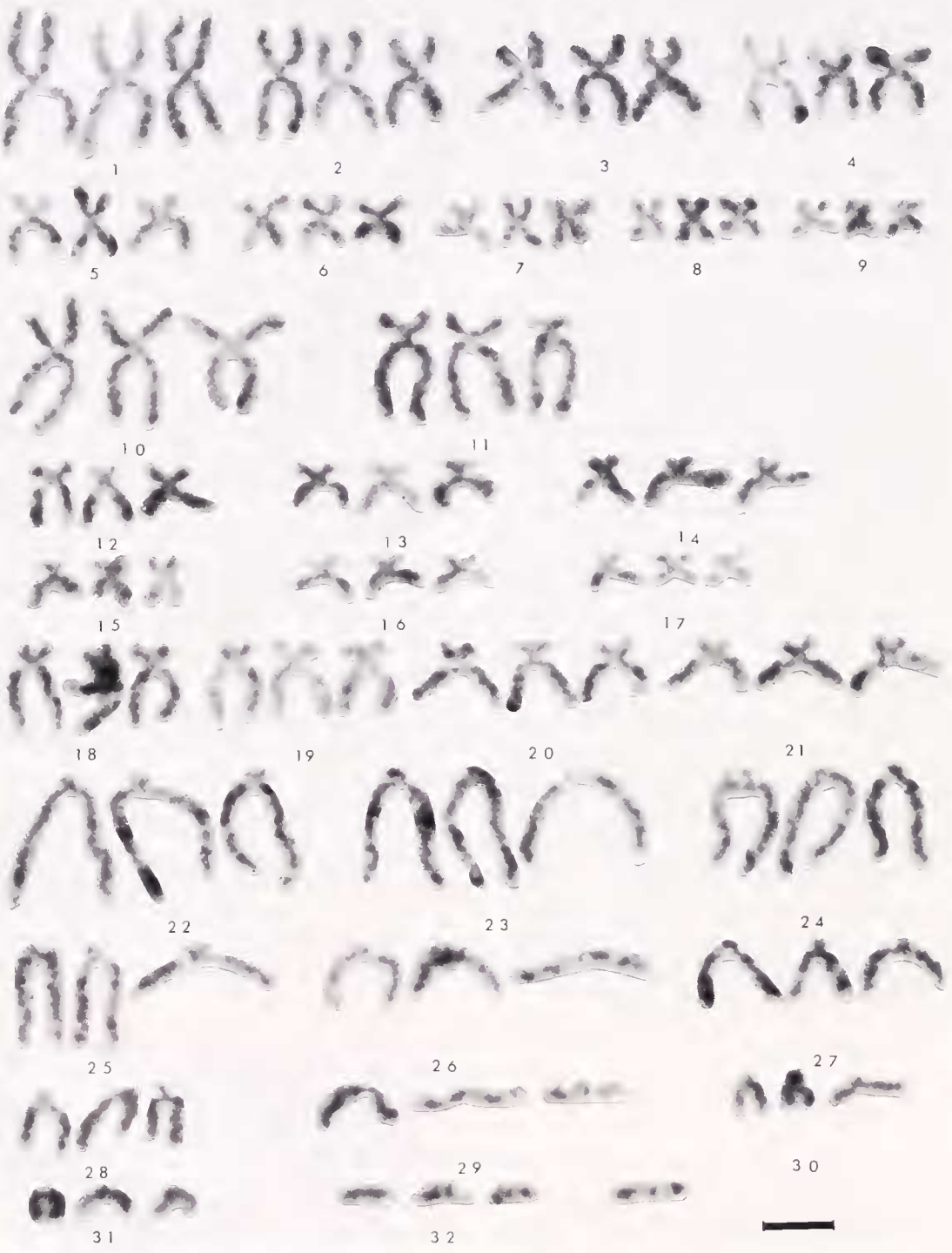


Figure 2. Karyotype taken from a northeastern Pacific *Lasaea* metaphase with 97 chromosomes. Scale bar = 5 μm.

ually fixed in three changes of freshly made Carnoy's fixitive (3:1 methanol, glacial acetic acid). Fixed broods were cleared in 45% glacial acetic acid and were gently pipetted into Vaseline-sealed wells on microscope slides. The cleared cells were viewed under phase-contrast or darkfield optics using a compound Zeiss photomicroscope and photographed with Kodak Tech Pan film. Cytological events following spawning were reconstructed

Table I

Chromosome measurements and classification in seven metaphases of northeastern Pacific Lasaea

Chromosome pair no.	Relative length		Arm ratio		Centromeric index		Classification
	Mean	SD	Mean	SD	Mean	SD	
1	5.75	0.16	0.864	0.037	46.18	1.06	m
2	4.69	0.28	0.757	0.066	42.85	2.05	m
3	3.98	0.21	0.733	0.056	42.07	1.77	m
4	3.51	0.33	0.777	0.078	43.92	2.42	m
5	2.63	0.33	0.811	0.075	44.43	2.30	m
6	2.38	0.31	0.842	0.069	45.46	1.97	m
7	2.11	0.39	0.873	0.056	46.37	1.54	m
8	1.92	0.33	0.821	0.087	44.75	2.64	m
9	1.62	0.26	0.842	0.082	45.45	2.52	m
10	5.89	0.55	0.436	0.063	29.91	3.00	m
11	4.50	0.37	0.423	0.056	29.42	2.86	sm
12	2.66	0.15	0.355	0.039	26.02	2.15	sm-st
13	2.47	0.20	0.353	0.079	25.70	4.14	sm-st
14	2.27	0.12	0.352	0.063	25.68	3.56	sm-st
15	2.16	0.22	0.366	0.067	26.41	3.43	sm-st
16	2.03	0.21	0.366	0.056	26.46	2.99	sm-st
17	1.80	0.22	0.346	0.075	25.27	4.05	sm-st
18	3.67	0.31	0.306	0.094	22.87	5.36	st-sm
19	3.28	0.22	0.328	0.038	24.32	1.98	st-sm
20	3.01	0.15	0.306	0.023	23.28	1.33	st
21	2.84	0.12	0.314	0.046	23.68	2.66	st
22	5.33	0.42	0.089	0.022	8.05	1.75	t
23	4.84	0.30	0.071	0.013	6.58	1.10	t
24	4.50	0.14	0.088	0.022	8.04	1.82	t
25	4.03	0.22	0.081	0.014	7.46	1.22	t
26	3.32	0.26	0.099	0.013	8.96	1.02	t
27	2.84	0.23	0.108	0.036	9.61	2.91	t
28	2.68	0.14	0.099	0.017	8.93	1.34	t
29	2.31	0.20	0.116	0.044	10.23	3.44	t
30	1.87	0.28	0.137	0.029	11.88	2.22	t-st
31	1.66	0.24	0.115	0.010	10.26	0.75	t
32	1.31	0.16	0.134	0.038	11.57	2.94	t-st

by examining broods that were fixed at different developmental stages.

Results

Karyology

The 85 slide preparations examined in this study contained large interphase nuclei (approximately 35 μm in diameter) and mitotic metaphase spreads. Meiotic divisions were not encountered. The mitotic metaphases were remarkable due to their large sizes and their high chromosome numbers (Fig. 1). Ninety-five metaphase spreads were photographed, and chromosome counts were scored, although the latter process was frequently complicated by the presence of overlapping chromosomes. Chromosome numbers ranged from 58 to 108; the majority of metaphases (76 out of 95) contained more than 80 chromosomes, and 32 spreads had 90–100 chromosomes.

Ten well-spread metaphases were karyotyped. Figure 2 shows the karyotype taken from one metaphase spread containing 97 chromosomes. The chromosomes have been arranged into 32 triplet subgroupings on the basis of shared morphology and size. Triplets can be further classified into groups of similar morphology: metacentrics, submetacentrics, subtelocentrics, and telocentrics. The identification of homologous chromosomes is relatively unambiguous for the largest chromosomes. But the smallest chromosomes have less distinctive morphologies, so ambiguities remain. One minichromosome within this metaphase (Fig. 2) could not be classified. The chromosomes of other karyotyped metaphases could similarly be arranged into triplets. Variation in chromosome number between metaphases resulted from the absence of single chromosomes from individual triplet subgroupings or from the occurrence of supernumerary chromosomes among the smaller members of each morphological group.

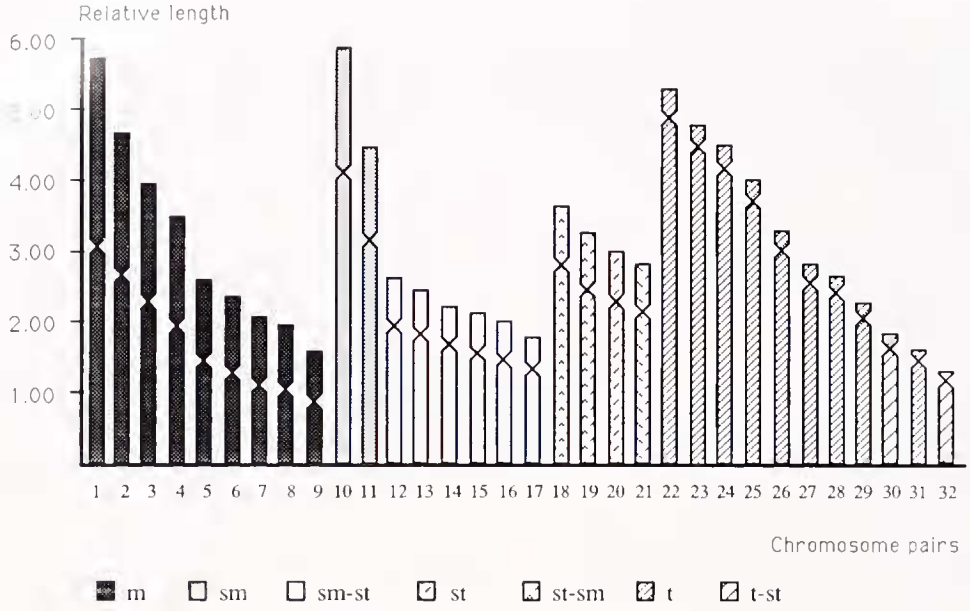


Figure 3. Ideogram of northeastern Pacific *Lasaea* chromosomes constructed from relative length and centromeric index values. Lengths are in μm. m, metacentric; sm, submetacentric; sm-st, submetacentric-subtelocentric; st, subtelocentric; st-sm, subtelocentric-submetacentric; t, telocentric; t-st, telocentric-subtelocentric.

Chromosomes were measured from seven well-spread metaphases (3 with 95 chromosomes, 3 with 97, and 1 with 100). Table I gives the mean and standard deviations of the relative lengths, arm ratios, and centromere indices of 32 chromosome triplets and their classification. An ideogram (Fig. 3) was constructed from the relative length and centromere index values so that the different morphological types of chromosomes could be better visualized.

The karyotype of northeastern Pacific *Lasaea* consists of 8 metacentrics, 8 submetacentrics (including 6 submetacentric-subtelocentrics), 4 subtelocentrics (including 2 subtelocentric-submetacentrics), and 11 telocentrics (including 2 telocentric-subtelocentrics). Within each morphological grouping of chromosomes, some triplets were difficult to distinguish because of their similar lengths: *e.g.*, metacentrics no. 7–8, submetacentric-subtelocentrics no. 12–13 and 15–16, subtelocentrics no. 20–21, and telocentrics no. 27–28 and 30–31. In these cases, the loss of individual chromosomes or the occurrence of supernumary chromosomes could not be assigned with accuracy.

Pronuclear interaction

A single sperm penetrates the cell membrane of each egg shortly after spawning. The egg germinal vesicle breaks down, and the egg chromosomes condense and become aligned for first metaphase (Fig. 4a). The incorporated sperm nucleus remains in the egg cortex in a condensed

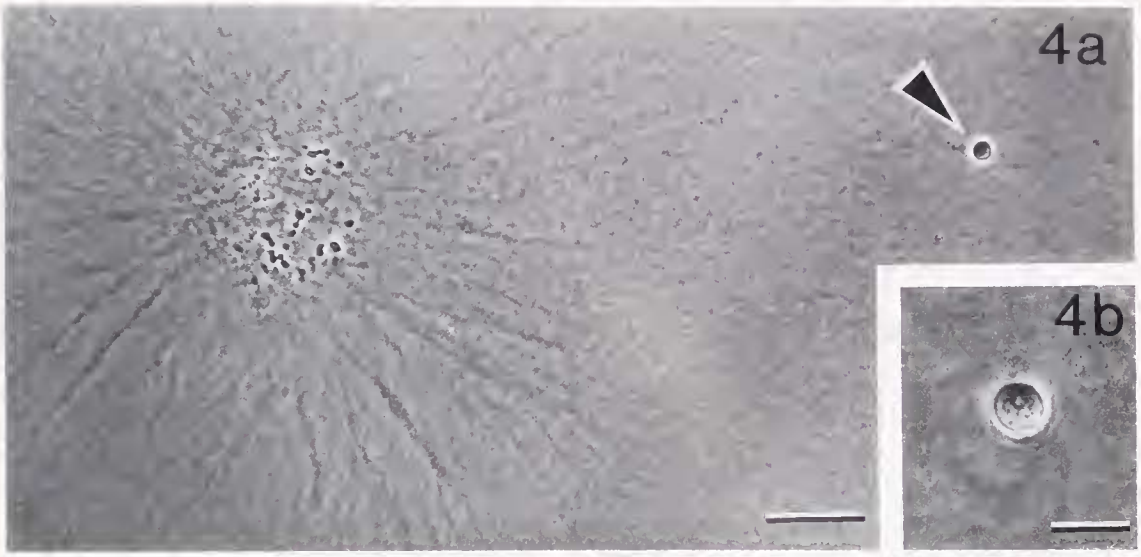
Figure 4a. Phase contrast light micrograph (PCLM) of an uncleaved northeastern Pacific *Lasaea* egg showing the incorporated, condensed sperm nucleus (arrow) in the cortex and the egg chromosomes arranged for first metaphase (on left). Scale bar = 20 μm.

Figure 4b. PCLM showing detail of sperm nucleus from Figure 4a. Scale bar = 6 μm.

Figure 5. PCLM of decondensing sperm nucleus (N) located in the cortex of an northeastern Pacific *Lasaea* egg. Scale bar = 10 μm.

Figure 6. Darkfield light micrograph (DFLM) of an uncleaved northeastern Pacific *Lasaea* egg with a decondensing sperm nucleus situated in its cortex (large arrow). This egg has extruded two polar bodies and the egg chromosomes are arranged for first cleavage metaphase (small arrow). Scale bar = 50 μm.

Figure 7. DFLM of a northeastern Pacific *Lasaea* first cleavage telophase. Note presence of aggregated sperm chromatin (n) in one of the daughter cells and of daughter chromosomes (large arrows) in both daughter cells. The cleavage furrow (c) is also apparent, as are the first (lower small arrow) and second (upper small arrow) polar bodies. Scale bar = 40 μm.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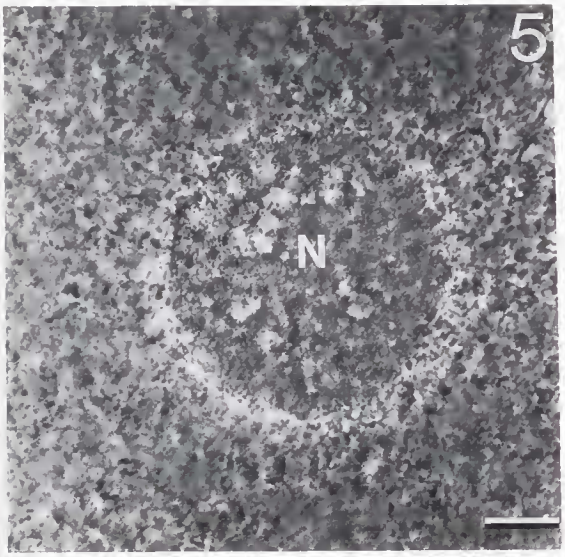
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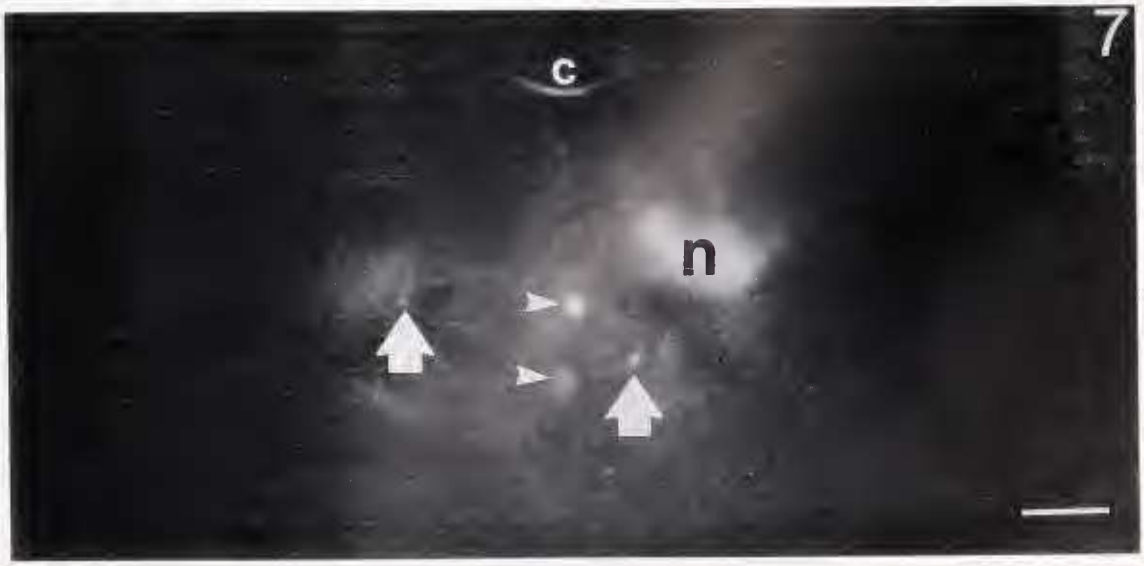
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c
n

state (Figs. 4a, b), until after the production of the two polar bodies. The sperm nucleus then slowly decondenses and can be distinctly visualized in the egg cortex with phase contrast (Fig. 5) and, especially, darkfield optics (Fig. 6). However, the sperm nucleus does not recondense to form chromosomes or to associate with the egg chromosomes. During first cleavage, the sperm chromatin remains aggregated and typically ends up in the cortex of one of the two daughter cells (Fig. 7).

Prior to first cleavage, the egg extrudes two polar bodies, each involving the formation of a spindle at the animal pole and the division of chromosomes. The first division (Fig. 8) results in the formation of a polar body containing an average of 48 (±5.2 S.E.; n = 17) well defined chromosomes (Fig. 9). After formation of an additional spindle, a second polar body is extruded adjacent to the first (Fig. 10). Unlike those of the first polar body, the chromosomes of the second are tightly aggregated and are very difficult to distinguish microscopically (Fig. 10). In a preliminary study, Ó Foighil (1987) assumed that the second polar body of northeastern Pacific *Lasaea* strains is haploid. During the present work, however, the second polar body chromatin was sufficiently dispersed in a few cases, and we could determine that each chromosome is composed of two homologous interphase chromatids (Fig. 11), *i.e.*, is diploid. In 2 eggs out of the 123 examined, the number of chromosomes in the second polar body could be established accurately and were in each case (49, 45) equal to that of the adjoining first polar body. The egg chromosomes that remain within the egg (female "pronucleus") migrate to the center of the cell and become arranged in homologous pairs for first cleavage (Fig. 12), without associating with the sperm nucleus. A mean of 95.9 (±12 S.E.; n = 9) chromosomes were arranged at the first cleavage metaphase. This is consistent with the results obtained in metaphase spreads of adult tissue. During first cleavage, the homologous chromosome pairs separate (Fig. 13) and migrate into the forming daughter cells.

Discussion

Northeastern Pacific *Lasaea* strains have high chromosome numbers, ploidy grouping of the chromosomes by three, and variable numbers of supernumerary chromosomes. Congeners that lack pelagic larvae have also been characterized karyologically from European (Thiriot-Quiévreux *et al.*, 1989) and from Kerguelen Island populations (Thiriot-Quiévreux *et al.*, 1988). These congeners share with northeastern Pacific strains the karyological features of high chromosome numbers and the presence of supernumerary chromosomes, but show varying degrees of polyploidy. In Kerguelen strains, a chromosome number of 100–120 was found, but ploidy levels could not be determined (Thiriot-Quiévreux *et al.*, 1988). Chromosome numbers in European strains range greatly (63–340) and can be grouped into different ploidy levels of 3, 5, and 6, in addition to variable supernumeraries (Thiriot-Quiévreux *et al.*, 1989).

A meaningful comparison of karyotypes among these different *Lasaea* populations is complicated because we cannot precisely distinguish homologous chromosome sets from the large and variable number of supernumaries. However, we can compare the first 17 chromosome pairs identified in the karyotype of Kerguelen strains (Thiriot-Quiévreux *et al.*, 1988) with the first 17 sets of homologous chromosomes ordered in decreasing size in the karyotypes of European (Thiriot-Quiévreux *et al.*, 1989) and of northeastern Pacific strains (this study). The karyotypes of the three populations differ in their relative numbers of metacentric, submetacentric, subtelocentric, and telocentric chromosome sets (8m, 3sm, 2st and 4t in Kerguelen strains; 6m, 1sm, 6st and 4t in European strains; 4m, 2sm, 4st and 7t in northeastern Pacific strains). These karyological distinctions not only imply that the three geographically isolated populations are reproductively incompatible, but also that they have experienced different evolutionary mechanisms of polyploidy.

Polyploidy in mollusks has been reported in several hermaphroditic pulmonate snails that are capable of self-fertilization or asexual reproduction (Jacob, 1957; Burch and Huber, 1966; Patterson and Burch, 1978; Goldman *et al.*, 1984). But among the Bivalvia, this phenomenon is exceptional, and beside the polyploid *Lasaea* strains, only one species, *Corbicula leana*, with a triploid chro-

Figure 8. PCLM showing a lateral view of the first metaphase spindle in a northeastern Pacific *Lasaea* egg. Scale bar = 8 μm.

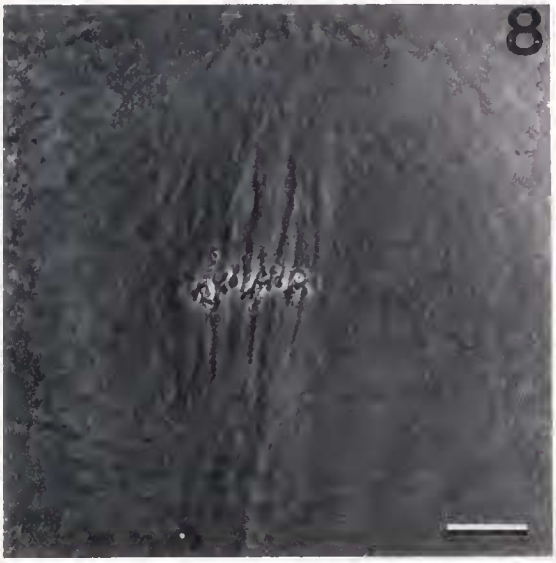
Figure 9. PCLM of the first polar body extruded by a northeastern Pacific *Lasaea* egg. Scale bar = 10 μm.

Figure 10. PCLM of both first (P) and second (arrow) northeastern Pacific *Lasaea* polar bodies. Scale bar = 20 μm.

Figure 11. PCLM showing details of northeastern Pacific *Lasaea* second polar body chromosomes. Arrows point to homologous chromatids. Scale bar = 7 μm.

Figure 12. PCLM of northeastern Pacific *Lasaea* egg chromosomes arranged at first cleavage metaphase. Arrows point to pairs of homologous chromosomes. Scale bar = 6 μm.

Figure 13. PCLM of first cleavage anaphase. Arrows indicate daughter chromosomes. Scale bar = 35 μm.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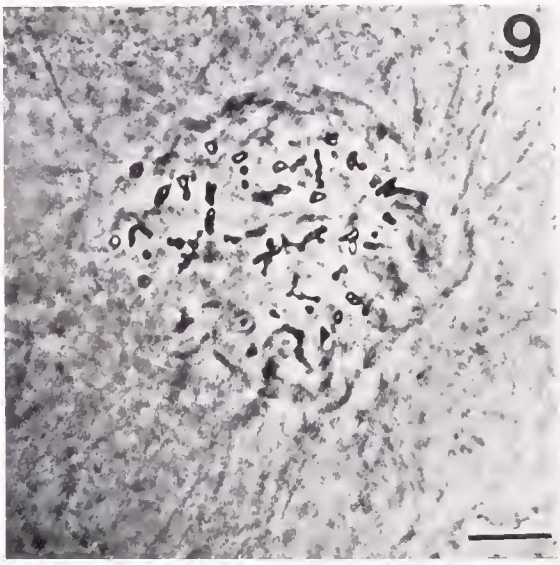
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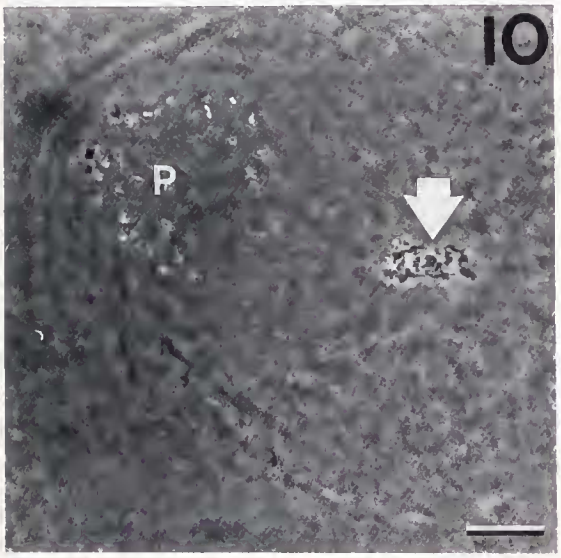
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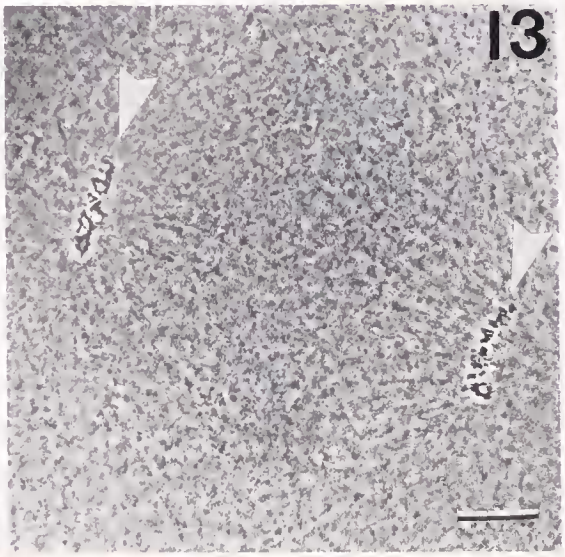
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mosome number of 54, has been recorded (Okamoto and Arimoto, 1986).

The genus *Lasaea* contains one sexual species, *L. australis*, that undergoes a planktotrophic larval development (Ó Foighil, 1988; Tyler-Walters and Crisp, 1989). Ó Foighil (1988) proposed that this reproductive and developmental combination represented the primitive condition in the genus. Tyler-Walters and Crisp (1989) provided a preliminary estimate of chromosome number in two *L. australis* eggs (one with n = 21–22, the other with 2n = 42–44). A more detailed karyological analysis currently underway has yielded a diploid number of 2n = 36 for this species (C. Thiriot-Quiévreux, unpubl.). Work in progress on the karyotype of *L. australis* may help to clarify chromosomal evolutionary mechanisms within the genus *Lasaea*.

Karyological analyses of *Lasaea* strains in both present and previous studies (Thiriot-Quiévreux *et al.*, 1988, 1989) have failed to find meiotic metaphases and the presence of odd ploidy numbers and of supernumary chromosomes presumably renders accurate meiosis impossible. Although not all aspects of egg maturation have been revealed in this present study, two key pieces of evidence establish that northeastern Pacific *Lasaea* strains are parthenogens, not self-fertilizers. The incorporated sperm nucleus disintegrates in the egg cortex and does not fuse with the egg "pronucleus"; *i.e.*, syngamy does not occur. Both polar bodies have a diploid chromosome number, a result inconsistent with meiosis, implying that they are products of mitotic divisions. Our data confirm Crisp and Standen's (1988) proposal that parthenogenetic development is triggered by auto sperm in *Lasaea* strains lacking dispersive larvae. Non-hybridizing lineages of northeastern Pacific *Lasaea* therefore represent true asexual clones, not inbred lines. *Lasaea* is the first bivalve genus in which asexual reproduction has been confirmed and is also the first molluscan genus in which pseudogamy (gynogenesis) has been detected.

Egg maturation in northeastern Pacific *Lasaea* differs from that of the great majority of other apomictic (ameiotic) organisms in that two polar bodies are extruded rather than one (Hughes, 1989). Two polar bodies are also mitotically produced in the gastropods *Thiara* (*Melanoides*) *tuberculatus* and *T. lineatus*, which avoid a reduction in chromosome number by arresting an oogonial division (Jacob, 1957). More detailed analyses of *Lasaea* egg maturation divisions are needed to determine if these organisms restitute chromosome numbers in a similar manner prior to first cleavage.

We have no direct data on the chromosome complement of the northeastern Pacific *Lasaea* sperm cells, but meiotic metaphases have not been discovered in *Lasaea* clones despite intensive karyological study of populations from British Columbia, Kerguelen Island, and Europe (present study, Thiriot-Quiévreux *et al.*, 1988, 1989). Reliance on sperm activation leaves open the possibility of occasional leakage of sperm chromosomes and may be the origin of the supernumary chromosomes that are characteristic of asexual *Lasaea* karyotypes.

Pseudogamy (gynogenesis) occurs in a wide variety of taxa (Kiester *et al.*, 1981; Stenseth *et al.*, 1985; Hughes, 1989) and pseudogamous individuals are typically sexual parasites of closely related cross-fertilizing species. *Lasaea* clones, however, are exceptional in that each individual is reproductively independent, using its own sperm to trigger asexual development. *Lasaea* clones, therefore, have much greater evolutionary potential than most gynogenetic forms.

Gynogens typically originate from hybridization events between related species, *e.g.*, the *Ambystoma jeffersonianum* complex (Uzzell, 1964), *Rana esculenta* (Uzzel and Berger, 1975; Turner and Nopp, 1979), and possibly the freshwater bivalve *Corbicula leana* (Okamoto and Arimoto, 1986). Hybrid, gynogenetic F1 progeny are frequently triploid, incapable of meiosis, and reproduce by sexually parasitizing males of the parental species (Hughes, 1989). Northeastern Pacific *Lasaea* clones are triploid and may have arisen from rare hybridization events between ancestral lineages. Hybrid, triploid *Lasaea* F1 progeny that produced a small amount of phenotypically normal sperm cells (Ó Foighil, 1985, 1987) may then have reproduced by autogynogenesis. This evolutionary scenario is, however, speculative and needs to be verified by independent phylogenetic reconstruction.

It is now apparent that the genus *Lasaea* includes some very unusual marine mollusks. Direct developing members are collectively much more successful than sexual congeners, which retain pelagic larval development (Ó Foighil, 1989). In the present study, we have confirmed that direct development in this genus is linked to polyploidy and to the presence of supernumary chromosomes (Thiriot-Quiévreux *et al.*, 1988, 1989). Direct development is also linked to minute male allocation (Pelseneer, 1903; Oldfield, 1964; Ó Foighil, 1985; McGrath and Ó Foighil, 1986; Ó Foighil and Eernisse, 1988) and to a clonal population genetic structure (Crisp *et al.*, 1983; Crisp and Standen, 1988; Ó Foighil and Eernisse, 1988; Tyler-Walters and Crisp, 1989). Autogynogenesis, which we have documented in northeastern Pacific *Lasaea* populations, may also be a persistent theme in other members of the genus that share this developmental mode, but see Tyler-Walters and Davenport (1990) for a potential exception. The genus *Lasaea* promises to become a valuable model system for exploring the long-term evolutionary and genetic consequences of contrasting reproductive modes in benthic marine metazoans.

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